

RESPONSES OF ORNL
Personnel to the EPA comments on
Technical Memorandum NO. 008
Bioindicator Assessment of Fish Health and Reproductive Success
in Lake Hartwell and Twelve Mile Creek

The following responses address the general and specific criticisms of the EPA reviewers, and follow the numbering system and format of that review. Specific responses were not generally provided in the case of numbered criticisms that required merely a simple change in the text (for example, the insertion of a missing reference). Criticisms that are not addressed in the following few pages may be assumed to have been corrected and/or satisfied.

General Comments:

- 1) In partial response to the reviewers' first general comment, two representative ORNL reports are provided. These also contain information on the back of their front covers detailing how interested parties can obtain other cleared reports through the proper channels. Also enclosed is a review on the bioindicator approach edited by one of the co-authors on this report. Unfortunately, a number of the more recent ORNL reports which would be most applicable to this Lake Hartwell report are not yet cleared for dissemination outside of DOE (however, this situation should change soon, as major efforts are underway to complete publication and clearance of these documents).
- 2) Procedures are explained to the degree that we felt was appropriate after taking into consideration many of the same factors that determine the amount of technical details presented in an open-literature publication. For instance, if a technique has been previously published in the open-literature, generally only deviations from the established routine or assay-specific details are described. An additional level of detail is presented if a procedure has been changed or adapted significantly from the original, if an established procedure is being used with a very different group of organisms (i.e. rodents versus fish), or if a previously unpublished technique is being presented for the first time.
In response to this criticism, we have included some additional detail in the Methods section where we thought it to be appropriate. In general, however, we believe that the level of detail in the Methods section is appropriate for this type of report.
- 3) We do not understand the reviewers' concerns as to the appropriateness of our measurements on largemouth bass and redbreast sunfish oocytes (see also response to specific comment no. 22). Largemouth bass and redbreast sunfish have oocytes that attain approximately the same size during development - a fact that is clearly shown in Figs. 13 and 28 of this report - so that there was no reason to adjust the oocyte measurements between species. We did have to adjust the way in which we estimated fecundity from these measurements based upon the differing patterns of clutch formation



in the two species, but these matters are already well discussed in the text.

- 4) The format of references has been standardized.

Specific Comments:

- 1) MS-222 might conceivably interfere with some blood analyses, but blood was drawn prior to the use of this agent.
- 2) This reference was inappropriate, and has been deleted.
- 3) "Appropriately diluted" is used in this case to mean that the sample was diluted so as to give results in a usable portion of a standard curve. This usage is standard for any procedure using such curves.
- 5) Personal communications are not commonly placed in a reference section.
- 7) Milt was thawed rapidly as appropriate for this type of assay.
- 9) Explained in text.
- 10) The text was correct. As stated in the text, the finding of "primary oocytes" in this testis was evidence of an abnormal situation (but not unprecedented among fish).
- 11) Homogenates were passed through an LH-20 column, eliminating potential interference by lipids in the actual assay.
- 12) Elsewhere in the review (see specific comment no. 8), the reviewers suggest that the specific locations of the intermediately-contaminated sites are not critical. We believe that the fact that the two sites were different (although close to each other in the lake) is significant and should be acknowledged, but should not preclude comparisons between the 1990 and 1992 results.
- 14) Section rewritten.
- 15) It was definitely *not* an oversight that bass were not collected for spawning purposes from the reference site. Originally, fish were to be collected for spawning purposes only if bass in spawning condition were available at the time of the primary fish collections for the project (please see SOW in Interagency Agreement for this project). Every possible attempt was made to coordinate the dates of this primary sampling effort with the time that the fish were expected to be spawning. However, as stated in the text of the report, spawning was delayed far past the expected spawning dates due to unseasonably cool water temperatures. Because of scheduling conflicts in other groups involved in this project, a decision was made to go ahead and conduct the primary fish sampling effort even though it was recognized that there was little, if any,

possibility that LMB would be spawning at the time (they weren't). In excess of the requirements of the SOW for this project, we agreed to return to Lake Hartwell later in the spring once COE personnel were able to verify that the bass were spawning. It was simply unfortunate that by the time we arrived at Lake Hartwell and had finished work at the contaminated study site, spawning activity at the reference site had apparently ceased.

16) Informal reference material such as this is not normally cited in the bibliography.

17) In perfect hindsight, Milwee Creek may not have been the most appropriate reference site for this type of study. However, it should be emphasized that Milwee Creek was a "reference site", not a "control site". It is nearly impossible to find a reference site that is itself not contaminated in some way (i.e. agricultural runoff). Having multiple reference sites is always preferable, since at least the range of responses to be expected in the absence of a particular source of environmental contamination (in this case, the PCB contamination of Twelve Mile Creek) can thus be documented. Additional reference sites were sampled for this study, but uncooperative weather conditions [again, in excess of the work called for in the SOW, we returned a large processing crew back to Lake Hartwell after having been rained-out during our first sampling attempt for this portion of the study by violent thunderstorms that made work in rain-swollen creeks totally impossible], scheduling conflicts, and an unanticipated absence of sufficient fish of the target species at some sites resulted in Milwee Creek being the only reference site for which we had a reasonable amount of bioindicator data.

18) See (12) above.

19) Now explained in text.

20) If by "damaged" the reviewer is referring to oocytes damaged during processing, the answer is "no". The misleading usage of the term "damaged" in this sentence has been deleted.

21) There were mistakes in this chart that have been corrected. We appreciate the reviewers identifying the inconsistencies.

22) This comment is very confusing. Clutch distribution based on oocyte size is readily apparent in the case of redbreast sunfish. In the case of bass, determining which oocytes are actually in a clutch is much more difficult; however, this is acknowledged in the text. As I pointed out in a recent presentation at the annual meeting of the American Fisheries Society, other methods used to estimate the fecundity of largemouth bass have much more serious flaws than the method employed in this study.

Editorial Comments (most were addressed by a simple but unacknowledged action):

1) Inserting commas in one of these instances (Twelve Mile Creek) would have

changed the meaning of the text.

13) Section rewritten.

40) Tables were left in their original order. This order is (and should be) determined by the order of appearance of a citation to the table in the text, and not by a wish for symmetry between two different sets of tables.